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(54) Title: ANTIGENIC IRON REPRESSIBLE PROTEINS FROM N. MENINGITIDIS RELATED TO THE HEMOLYSIN FAMILY OF TOXINS

(57) Abstract

An isolated, antigenic polypeptide comprises a segment having at least fifty amino acid residues. The amino acid sequence of the segment is present in *N. meningitidis*, and is different from, but substantially homologous with, the amino acid sequence of a segment of a member of the hemolysin family of toxins.

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ANTIGENIC IRON REPRESSIBLE PROTEINS FROM N. MENINGITIDIS RELATED TO THE HEMOLYSIN FAMILY OF TOXINS

The present invention is directed to antigenic polypeptides isolated from Neisseria meningitidis, antibodies raised against the polypeptides, vaccines containing the polypeptides and DNA encoding the polypeptides. The polypeptides are members of the hemolysin family of toxins, a typical member of which is alpha-hemolysin from E. coli.

Bacterial pathogenesis is a complicated and often poorly understood process. Many pathogenic bacteria secrete toxins that impair the metabolism and function of animal cells. Various classes of molecules constitute such toxins.

An example of a protein toxin is found in pathogenic E. coli strains that cause extra-intestinal infections in humans. Such infections are characterized by the lysis of mammalian erythrocytes. The hemolytic activity is due to a class of toxins known as hemolysin. The class includes alpha-hemolysin and beta-hemolysin; see Welch et al, Infection and Immunity 42, 178-186 (1983).

Another protein toxin is adenylate cyclase, which is found in Bordetella pertussis and Bacillus anthracis, and which impairs functions of professional phagocytes. These bacteria are responsible for whooping cough and anthrax, respectively.

A third class of protein toxins from pathogenic bacteria are the leukotoxins, which are found in Actinobacillus actinomycetemcomitans, which is the etiologic agent of localized juvenile periodontitis, and Pasteurella haemolytica, which kills bovine leukocytes.

Interestingly, the adenylate cyclase from B. pertussis and B. anthracis and the leukotoxins from A. actinomycetemcomitans and P. haemolytica have amino acid sequences that exhibit considerable homology with that of alpha-hemolysin from E. Coli; see Glaser et al, Molecular Biology 2, 19-30 (1988) and Kolodrubetz et al, Infection and Immunity 57, 1465-1469 (1989). Apparently, there is a class of toxins found in various genera of bacteria. The amino acid sequence of this family of cytotoxins is characterized by a highly repeated nine amino acid motif, LxGGxGNDx, wherein x represents any amino acid. For the purposes of this specification, this family of toxins will be referred to as the hemolysin family of toxins.

It should be understood that "hemolysin family of toxins" is a generic name familiar to those in the art, and is not meant to imply that all members are hemolytic, or, for that matter, cytotoxic, although most are. Membership in the family depends on the existence of homology in the amino acid sequence, as defined below. In addition to those mentioned above, homologous proteins have also been found in Serratia and Proteus, although it is not certain whether these members of the hemolysin family are, in fact, cytotoxic.

Little is known about the intriguing and sometimes fatal bacteria Neisseria meningitidis, which is responsible for spinal meningitis and septic shock. N. meningitidis and the diseases it causes have been reviewed by Paterson in "Neisseria meningitidis and Meningococcal Disease" in Biologic and Clinical Basis of Infectious Diseases, W. B. Saunders Company, Chapter 43 (1980).

The genotype of N. meningitidis is very similar to that of N. gonorrhoeae, although the phenotype is quite different. It is often important to distinguish between these Neisseria species. Immunologic speciation is often difficult due to a lack of sufficient amounts of group-specific antigens.

N. meningitidis exists as various serotypes, the prevalence of which varies with time and location. The serotypes include A, B, C, D, X, Y, Z, 29-E and W-135.

The three most important known antigenic and/or toxic constituents of N. meningitidis infections are a capsular polysaccharide, a lipopolysaccharide-endotoxin cell wall complex and a Neisseria-specific protein. The capsular polysaccharide is a major _virulence factor that enables meningococci to resist phagocytosis by segmented neutrophils.

Vaccines containing meningococcal polysaccharides are used against some of the serotypes of N. meningitidis. For example, protection against the A, C, Y and W-135 serotypes is afforded by polysaccharide vaccines. Such vaccines are, however, inadequate for general protection against infection against N. meningitidis. For example, the immune response of serotypes A and C to polysaccharide vaccines is poor, especially in children under two years old, who constitute the group most susceptible to meningococcal disease. Moreover, no effective vaccine exists for serotype B, possibly because the group B capsular polysaccharide is relatively non-immunogenic.

It is apparent that much needs to be learned about the pathology of N. meningitidis. Possibly, additional understanding of this pathogen will lead to the discovery of useful vaccines in general for more serotypes than are currently available.

For example, it is not known why the colonization of the respiratory tract by N. meningitidis progresses to acute meningococcal disease and sometimes death in an occasional individual, whereas it does not do so in the great majority of others who are apparently at comparable risk. The amount of neither capsular polysaccharide nor lipopolysaccharide-endotoxin complex correlates with the seriousness of this disease. Exposure to microorganisms with antigenic constituents that cross-react with capsular polysaccharides of N. meningitidis has been proposed as an explanation; see Paterson, id.

Other explanations are also possible. For example, cross-immunity to antigens other than capsular polysaccharides cannot be ruled out. It is interesting to note in this regard that there are no known protein toxins associated with N. meningitidis. One reason for this may be that N. meningitidis is often cultured in vitro under iron-rich conditions that do not exist in a human host.

It is known, however, that some meningococcal proteins are iron-repressed and are not observed in vitro, although they are expressed in vivo. See Black et al., Infection and Immunity 54, 710-713 (1986) and Brener et al, ibid. 33, 59-66 (1981).

One problem addressed by the present invention is the discovery of antigenic polypeptides and DNA sequences that are capable of identifying N. meningitidis and distinguishing it from N. gonorrhoeae. Another problem addressed by the present invention is the discovery of proteins capable of producing antibodies effective against meningococcal disease.

SUMMARY OF THE INVENTION

These and other problems as will be apparent to those having ordinary skill in the art have been solved by providing an isolated, antigenic polypeptide comprising a segment having at least fifty amino acid residues, wherein the amino acid sequence of the segment is present in N. meningitidis, and wherein the amino acid sequence is different from, but substantially homologous with, the amino acid sequence of a segment of a member of the hemolysin family of toxins.

Another way of defining the polypeptide is to say that it is an isolat d polypeptide comprising a segment having an amino acid sequence present in N. meningitidis wherein the amino acid sequence consists of at least three repeats of the nine amino acid hemolysin consensus sequence, the hemolysin consensus sequence consisting of at least four of:

L at position 1;

G at position 3;

G at position 4;

G at position 6;

N at position 7;

D at position 8; and

x at positions 2, 5 and 9;

wherein x, independently, represents any single amino acid residue.

The invention further includes antigenic fragments of such polypeptides, antibodies raised against such polypeptides, nucleotide sequences encoding such polypeptides, and vaccines containing such polypeptides.

DETAILED DESCRIPTION OF THE INVENTION

The Polypeptide Segment

It has unexpectedly been discovered that when N. meningitidis is grown under iron-limiting conditions, a

polypeptide comprising a segment having an amino acid sequence that is different from, but substantially homologous with, a segment of the hemolysin family of toxins is expressed. Monoclonal antibodies raised against the polypeptide found in N. meningitidis, such as A4.85 (see below), cross-react in Western blots with alpha-hemolysin (HlyA) produced by the hlyA gene in E. coli and adenylate cyclase produced in Bordetella pertusssis.

The hemolysin family of toxins, as used herein, includes the homologous, cytotoxic or proteolytic polypeptides found in bacteria of the genera Escherichia, Serratia, Pasteurella, Proteus, Actinobacillus, and Bordetella. The family specifically includes alpha-hemolysin, leukotoxin, and adenylate cyclase.

Determinations whether two amino acid sequences are substantially homologous are, for the purpose of the present specification, based on FASTA searches in accordance with Pearson and Lipman, Proc. Natl. Acad. Sci. USA 85, 2444-2448 (1988). A substantially homologous sequence in accordance with the present invention has at least 15% identity, preferably at least 20% identity and, more preferably, at least 25% identity in amino acid sequence when determined in accordance with the method of

Pearson and Lipman, which is incorporated herein by reference.

The polypeptide of the present invention need not contain a segment that is identical to other members of the hemolysin family of toxins. The identity in accordance with the FASTA method may be as high as 90% or 95%, but when isolated from N. meningitidis normally does not exhibit identities greater than 40% or 50%.

The size of the polypeptide is not critical, as long as it contains a segment that is substantially homologous to a segment of a member of the hemolysin family of toxins. The substantially homologous segment has at least fifty amino acid residues, preferably at least 100 amino acid residues, and more preferably at least 200 amino acid residues. In this specification, the word "polypeptide" will be considered indistinguishable from words like "protein" and "peptide."

The segment of the meningococcal polypeptide that is substantially homologous to a segment of the hemolysin family of toxins contains the same nine amino acid motif that is characteristic of all the members of the hemolysin family of toxins. The consensus sequence is LxGGxGNDx,

hereinafter hemolysin consensus sequence. The amino acid represented by x may be any single amino acid.

The polypeptide of the invention may be defined in terms of the hemolysin consensus sequence as well as by the substantial homology standard described above. For this purpose, a nine amino acid sequence is considered to be a hemolysin consensus sequence if it contains at least four, preferably at least five, and more preferably all six of the specifically defined amino acid residues (i.e. L-GG-GND-) at the correct position.

Referring to Figure 2, which, as described below, is a partial polypeptide isolated from N. meningitidis, the homologous segment comprises three stretches of multiple repeats of the hemolysin consensus sequence, i.e. between amino acids 486 and 512, between amino acids 623 and 712, and from 823 to the end. There are 21 complete consensus sequences in Figure 2.

The polypeptides of the present invention contain segments that are present in N. meningitidis and that comprise at least three, preferably at least five, and more preferably at least ten hemolysin consensus sequences. The polypeptides of the invention may have as many as at least 21 hemolysin consensus sequences.

The polypeptide is isolated, which means that it is essentially free of other proteins, especially of other proteins from N. meningitidis. Essentially free from other proteins means that it is at least 90%, preferably at least 95% and, more preferably, at least 98% free of other proteins.

Preferably, the polypeptide is essentially pure, which means that the polypeptide is free not only of other polypeptides, but also of other materials used in the isolation and identification of the polypeptide, such as, for example, sodium dodecyl sulfate and other detergents as well as nitrocellulose paper. The polypeptide is at least 90% free, preferably at least 95% free and, more preferably, at least 98% free of such materials.

The polypeptide of the present invention is antigenic, which means that the polypeptide induces specific antibodies in a mammal. Preferably, the polypeptide is immunogenic.

The polypeptide may be the entire polypeptide as it exists in N. meningitidis, or an antigenic, preferably immunogenic, fragment of the whole polypeptide. Antigenic and/or immunogenic fragments of antigenic and/or immunogenic polypeptides may be identified by methods

known in the art. Usually, the antigenic fragment will comprise at least a portion of the segment having an amino acid sequence that is different from, but homologous, to the amino acid sequence of a segment of a polypeptide that is a member of the hemolysin family of toxins, or will comprise at least a portion of the segment having at least three, preferably at least five, and more preferably at least ten hemolysin consensus sequences.

Preparation of the Polypeptide

The polypeptides of the present invention may be prepared by methods known in the art. Such methods include isolating the polypeptide directly from N. meningitidis; isolating or synthesizing DNA encoding the polypeptide and using the DNA to produce recombinant polypeptide; and synthesizing the polypeptide from individual amino acids.

The polypeptide or DNA encoding the polypeptide may be isolated from any serotype of N. meningitidis. Such serotypes include A, B, C, D, X, Y, Z, 29-E and W-135.

Suitable sources of meningococcal strains from which the polypeptide and DNA encoding the polypeptide may be isolated are available. Such sources include the American

Type Culture Collection (Bethesda, MD) and the Neisseria Repository (NAMRU, University of California, Berkeley). Suitable strains include FAM18 and FAM20 (Dyer et al, Microbial Pathogenesis 3, 351-363 (1987)), and FAM 19. Additional meningococcal strains are described by Schryvers and Morris in Infection and Immunity 56, 1144-1149 (1988).

The polypeptide may be isolated directly from N. meningitidis by methods known in the art. First, meningococcal outer membranes are isolated and prepared by known methods. The methods described by West and Sparling in Infect. Immun. 47, 388-394 (1985) and by Schryvers and Morris in Infect. Immun. 56, 1144-1149 (1988) are suitable.

The isolated membrane proteins may be solubilized by known methods, such as the addition of detergents. Commonly used detergents include Octyl-B-Glucoside, Chaps, Zwittergent 3.14 or Triton-X. The use of detergents to enhance solubility of membrane proteins is described by Jones et al. in Finby, Solubilization and Reconstitution of Membrane Proteins: A Practical Approach, IRL Press (1986), Helenius et al. in Biochim. Biophys. Acta 415, 29 (1975) and Hjelmeland and Chrambach, Methods Enzymol. 104, 305 (1984).

Proteins are isolated from the solubilized membrane fraction by standard methods. Some suitable methods include precipitation and liquid chromatographic protocols such as ion exchange, hydrophobic interaction and gel filtration. See, for example, Methods Enzymol. 182 (Guide to Protein Chemistry, Deutscher, Ed. Section VII) 309 (1990) and Scopes, Protein Purification. Springer-Verlag, New York (1987).

Alternatively, purified material is obtained by separating the protein on preparative SDS-PAGE gels, slicing out the band of interest and electroeluting the protein from the polyacrylamide matrix by methods known in the art. The detergent SDS is removed from the protein by

known methods, such as by dialysis or the use of a suitable column, such as the Extracti-Gel column from Pierce.

The polypeptide may also be produced by isolating DNA that encodes the polypeptide; cloning the DNA in a suitable host; expressing the DNA in the host; and harvesting the polypeptide.

The first DNA encoding the polypeptide of the present invention was isolated by an immunoscreening method. Such methods are described by Maniatis et al in "Molecular Cloning: A Laboratory Manual," Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1982).

Briefly, monoclonal antibodies were generated against iron-stressed outer membrane proteins of N. meningitidis strain FAM20. One monoclonal antibody, A4.85, recognized several iron-regulated proteins in Western blots of the FAM20 outer membranes. A4.85 was used to isolate a clone from an FAM20 genomic library constructed in the expression vector lambda-gt11. The sequence of this clone was determined and used to clone adjacent genomic restriction fragments. The adjoined DNA sequence of this region contained a long open reading frame, which is shown

as Figure 1. The amino acid sequence predicted from the nucleotide sequence of Figure 1 is shown as Figure 2.

A FASTA homology search in accordance with Pearson and Lipman, Proc. Natl. Acad. Sci. USA 85, 2444-2448 (1988) of the amino acid sequence deduced from the open reading frame (Figure 2) was performed. Surprisingly, the amino acid sequence exhibited substantial homology to several members of the hemolysin family of toxins, as discussed above.

As further evidence that the polypeptide isolated from N. meningitidis is a member of the hemolysin family of toxins, the antibody raised against, and used to isolate, the polypeptide shown as Figure 2, A4.85, cross-reacted strongly with alpha-hemolysin (HlyA) from E. coli and with adenylate cyclase produced in Bordetella pertusssis.

The immunoscreening method may be repeated in order to obtain additional fragments of the gene encoding the polypeptide of the invention or to obtain the gene encoding the entire polypeptide. It is, of course, not necessary to repeat the immunoscreening process. The entire gene or additional fragments of the gene are preferably isolated by using the known DNA sequence or

fragments thereof as a probe. To do so, meningococcal DNA restriction fragments, either flanking the ends of the region already cloned or containing the entire region, are identified by Southern hybridization using labelled oligonucleotide probes derived from a previously determined sequence, such as that shown as Figure 1, or a fragment thereof.

The DNA obtained may be amplified by methods known in the art. One suitable method is the polymerase chain reaction (PCR) method described by Mullis et al in U.S. Patent 4,683,195 and by Sambrook, Fritsch and Maniatis (eds) in Molecular Cloning, A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press (1989). It is convenient to amplify the clones in the lambda-gt11 vectors using lambda-gt11-specific oligomers as the amplimers (available from Stratagene).

The restriction fragments are cloned into a suitable vector, such as a plasmid or bacteriophage, and sequenced in accordance with methods known in the art. A suitable sequencing method is the dideoxy chain terminating method described by Sanger et al in Proc. Natl. Acad. Sci. USA 74, 5463-5467 (1977). Suitable vectors and polymerases for sequencing are known. A suitable vector is the Bluescript vector of Stratagene. A suitable polymerase is

Sequenase (United States Biochemical Corp., Cleveland, OH).

The DNA encoding the polypeptide of the invention may be used to express recombinant polypeptide in a wide variety of host cells using a wide variety of vectors. The host may be prokaryotic or eukaryotic. The DNA may be obtained from natural sources and, optionally, modified. The genes may also be synthesized in whole or in part.

Cloning vectors may comprise segments of chromosomal, non-chromosomal and synthetic DNA sequences. Some suitable prokaryotic vectors include plasmids from E. coli, such as colE1, pCR1, pBR322, pMB9, and RP4. Prokaryotic vectors also include derivatives of phage DNA such as M13, fd, and other filamentous single-stranded DNA phages.

Vectors for expressing proteins in bacteria, especially E.coli, are also known. Such vectors include pK233 (or any of the tac family of plasmids), T7, and lambda P_L. Examples of vectors that express fusion proteins include the PATH vectors described by Dieckmann and Tzagoloff in J. Biol. Chem. 260, 1513-1520 (1985). These vectors contain DNA sequences that encode anthranilate synthetase (TrpE) followed by a polylinker at

the carboxy terminus. Other expressi n vector systems are based on beta-galactosidase (pEX); maltose binding protein (pMAL); and glutathione S-transferase (pGST) - see Gene 67, 31 (1988) and Peptide Research 3, 167 (1990).

Vectors useful in yeast are available. A suitable example is the 2u plasmid.

Suitable vectors for use in mammalian cells are also known. Such vectors include well-known derivatives of SV-40, adenovirus, retrovirus-derived DNA sequences and vectors derived from combination of plasmids and phage DNA.

Further eukaryotic expression vectors are known in the art (e.g., P.J. Southern and P. Berg, J. Mol. Appl. Genet. 1, 327-341 (1982); S. Subramani et al, Mol. Cell. Biol. 1, 854-864 (1981); R.J. Kaufmann and P.A. Sharp, "Amplification And Expression Of Sequences Cotransfected with A Modular Dihydrofolate Reductase Complementary DNA Gene," J. Mol. Biol. 159, 601-621 (1982); R.J. Kaufmann and P.A. Sharp, Mol. Cell. Biol. 159, 601-664 (1982); S.I. Scahill et al, "Expression And Characterization Of The Product Of A Human Immune Interferon DNA Gene In Chinese Hamster Ovary Cells," Proc. Natl. Acad. Sci. USA 80, 4654-

4659 (1983); G. Urlaub and L.A. Chasin, Proc. Natl. Acad. Sci. USA 77, 4216-4220, (1980).

Useful expression hosts include well-known prokaryotic and eukaryotic cells. Some suitable prokaryotic hosts include, for example, E. coli, such as E. coli SG-936, E. coli HB 101, E. coli W3110, E. coli X1776, E. coli X2282, E. coli DHI, and E. coli MRCl, Pseudomonas, Bacillus, such as Bacillus subtilis, and Streptomyces. Suitable eukaryotic cells include yeasts and other fungi, insect, animal cells, such as COS cells and CHO cells, human cells and plant cells in tissue culture.

The expression vectors useful in the present invention contain at least one expression control sequence that is operatively linked to the DNA sequence or fragment to be expressed. The control sequence is inserted in the vector in order to control and to regulate the expression of the cloned DNA sequence. Examples of useful expression control sequences are the lac system, the trp system, the tac system, the trc system, major operator and promoter regions of phage lambda, the control region of fd coat protein, the glycolytic promoters of yeast, e.g., the promoter for 3-phosphoglycerate kinase, the promoters of yeast acid phosphatase, e.g., Pho5, the promoters of the

yeast alpha-mating factors, and promoters derived from polyoma, adenovirus, retrovirus, and simian virus, e.g., the early and late promoters or SV40, and other sequences known to control the expression of genes of prokaryotic or eukaryotic cells and their viruses or combinations thereof.

The recombinant polypeptide is purified by methods known in the art. Suitable methods are described F. A. O. Marston, "The Purification of Eukaryotic Polypeptides Expressed in Escherichia coli," in DNA Cloning, D. M. Glover, Ed., Vol. III, IRL Press Limited, England (1987).

The polypeptide of the invention and DNA encoding the polypeptide may also be chemically synthesized from individual amino acid residues and nucleotides, respectively, by methods known in the art. Suitable methods for synthesizing the polypeptide are described by Stuart and Young in "Solid Phase Peptide Synthesis," Second Edition, Pierce Chemical Company (1984). Suitable methods for synthesizing DNA are described by Caruthers in Science 230, 281-285 (1985).

VACCINES

A polypeptide comprising a segment having an amino acid sequence that is different from, but substantially

homologous with, th amino acid sequence of a member of the hemolysin family of toxins is, unexpectedly, an antigen useful for protecting a mammal from infectious diseases caused by N. meningitidis. The mammal is typically a human.

To be useful, the antigen is non-toxic to the mammal being immunized. If the antigen is toxic, it may be detoxified by methods known in the art. Such methods include, for example, providing antigenic, non-toxic fragments of the entire polypeptide or detoxifying a polypeptide by, for example, binding the toxin to a carrier molecule that destroys toxicity, but does not affect antigenicity. The carrier molecule is typically another polypeptide.

Preferably, an amino acid sequence of the antigen is present in a polypeptide found in N. meningitidis. The polypeptide or non-toxic, antigenic fragments useful in immunizing mammals may be made by methods known in the art, such as by isolation from N. meningitidis, production by recombinant DNA techniques, or chemical synthesis, as described above.

The length of the fragment is not critical as long as the fragment is antigenic and non-toxic. Therefore, the

fragment should contain sufficient amino acid residues to define the epitope. Methods for isolating and identifying antigenic fragments from known antigenic polypeptides are described by Salfeld et al. in J. Virol. 63, 798-808 (1989) and by Isola et al. in J. Virol. 63, 2325-2334 (1989).

If the fragment defines the epitope, but is too short to be antigenic, it may be conjugated to a carrier molecule. Some suitable carrier molecules include keyhole limpet hemocyanin and bovine serum albumen. Conjugation may be carried out by methods known in the art. One such method is to combine a cysteine residue of the fragment with a cysteine residue on the carrier molecule.

The present invention further includes vaccine compositions for immunizing mammals, including humans, against infection by N. meningitidis. The vaccine comprises an immunogenic antigen as described above in a suitable carrier. Suitable carriers include any of the standard pharmaceutically acceptable carriers, such as water, phosphate buffered saline solution, and emulsions.

The vaccine may include adjuvants, such as muramyl peptides, and lymphokines, such as interferon,

interleukin-1 and interleukin-6. The antigen may be adsorbed on suitable particles, such as aluminum oxide particles, or encapsulated in liposomes, as is known in the art.

The invention further includes methods of immunizing host mammals, including humans, by administering the vaccine compositions described above to mammals in need of protection from diseases caused by N. meningitidis. The vaccine comprises an immunogenic polypeptide in a form that is non-toxic to mammals. The polypeptide comprises an amino acid sequence that is homologous with the amino acid sequence of a member of the hemolysin family of toxins. The amino acid sequence is preferably present in N. meningitidis, and is usually found in the outer membranes of N. meningitidis. Since, however, antibodies cross-react with the polypeptide of the invention and members of the hemolysin family of toxins from other genera of bacteria, the antigen in the vaccine composition may comprise an amino acid sequence in such other genera, such as from E. coli or B. pertussis.

The vaccine may be administered to a mammal by methods known in the art. Such methods include, for example, intravenous, intraperitoneal, subcutaneous, or intramuscular administration.

Antibodies

The present invention provides antibodies raised against a polypeptide of the invention. The polypeptide comprises an amino acid sequence that defines an epitope, and is substantially homologous with the amino acid sequence of a member of the hemolysin family of toxins. The antibodies are preferably raised against a polypeptide comprising an amino acid sequence that is present in N. meningitidis, and that is different from polypeptides that are members of the hemolysin family of toxins from other genera of bacteria.

The antibodies are preferably monoclonal. Monoclonal antibodies may be produced by methods known in the art. These methods include the immunological method described by Kohler and Milstein in Nature 256, 495-497 (1975) and the recombinant DNA method described by Huse et al in Science 246, 1275-1281 (1989).

Mammals, including humans, suffering from diseases caused by infection with N. meningitidis may be treated by administering antibodies specific to a member of the hemolysin family of toxins. Antibodies raised against a member of the hemolysin family of toxins from any genera of bacteria are suitable, although antibodies raised

against a polypeptide comprising an amino acid sequence present in N. meningitidis is preferred.

For therapeutic purposes, it is necessary for the antigenic polypeptides of the invention to produce neutralizing antibodies. Neutralizing antibodies are antibodies that significantly inhibit the growth of or kill the bacterial cells and/or significantly neutralize the toxin function of the polypeptide in vitro or in vivo. Growth of the bacteria is significantly inhibited or the toxin function of the polypeptide is significantly neutralized in vivo if the inhibition or neutralization is sufficient to prevent or reduce the symptoms of the disease of a mammal infected with the disease.

Neutralizing antibodies may also be used to produce anti-idiotypic antibodies useful as vaccines for immunizing mammals, including humans, suffering from diseases caused by infection with N. meningitidis. Anti-idiotypic antibodies are prepared in accordance with methods known in the art.

NUCLEIC ACID MOLECULES

The present invention also includes isolated nucleic acid molecules that encode any of the polypeptides of the

invention described above. The nucleic acid molecule may be DNA or RNA.

The utility of the nucleic acid molecule lies in its ability to be used as a probe for detecting N. meningitidis, as explained below, or to produce a polypeptide of the invention, as explained above. The nucleic acid molecule may be prepared by methods known in the art. Suitable methods include isolating the DNA from N. meningitidis or synthesizing the DNA in accordance with known procedures as described above.

PROBES

The present invention further provides a method of detecting the presence of N. meningitidis in a sample. The method involves use of a probe that recognizes a polypeptide that is a member of the hemolysin family of toxins, and, in particular, a member of the hemolysin family of toxins present in N. meningitidis, or a gene encoding such a polypeptide. The probe recognizes N. meningitidis if present in the sample.

The probe may be an antibody, preferably a monoclonal antibody. The antibodies may be prepared as described above.

Methods are known for detecting polypeptides with antibodies. For example, a polypeptide may be immobilized on a solid support. Immobilization of the polypeptide may occur through an immobilized first antibody specific for the polypeptide. The immobilized first antibody is incubated with a sample suspected of containing the polypeptide. If present, the polypeptide binds to the first antibody.

A second antibody, also specific for the polypeptide, binds to the immobilized polypeptide. The second antibody may be labelled by methods known in the art. Non-immobilized materials are washed away, and the presence of immobilized label indicates the presence of the polypeptide. This and other immunoassays are described by David, et al., in U.S. Patent 4,376,110 assigned to Hybritech, Inc., La Jolla, California.

The probe may also be a nucleic acid molecule that recognizes a RNA or DNA molecule that encodes a member of the hemolysin family of toxins present in N. meningitidis. Methods for determining whether a nucleic acid molecule probe recognizes a specific nucleic acid molecule in a sample are known in the art. Generally, a labelled probe that is complementary to a nucleic acid sequence suspected of being in a sample is prepared. The presence of probe

hybridized to th target nucleic acid molecule indicates the presence of the nucleic acid molecule. Suitable methods are described by Schneider et al in U.S. Patent 4,882,269, which is assigned to Princeton University, and by Segev in PCT Application WO 90/01069. The Schneider et al patent and the Segev application are both licensed to ImClone Systems Inc., New York City.

The probes described above are labelled in accordance with methods known in the art. Methods for labelling antibodies have been described, for example, by Hunter and Greenwood in Nature 144, 945 (1962) and by David et al in Biochemistry 13, 1014-1021 (1974). Additional methods for labelling antibodies have been described in U.S. patents 3,940,475 and 3,645,090. Methods for labelling oligonucleotide probes have been described, for example, by Leary et al, Proc. Natl. Acad. Sci. USA (1983) 80:4045; Renz and Kurz, Nucl. Acids Res. (1984) 12:3435; Richardson and Gumport, Nucl. Acids Res. (1983) 11:6167; Smith et al, Nucl. Acids Res. (1985) 13:2399; and Meinkoth and Wahl, Anal. Biochem. (1984) 138:267.

The label may be radioactive. Some examples of useful radioactive labels include ^{32}P, ^{125}I, ^{131}I, and ^{3}H. Use of radioactive labels have been described in U.K. 2,034,323, U.S. 4,358,535, and U.S. 4,302,204.

Some examples of non-radioactive labels include enzymes, chromophors, atoms and molecules detectable by electron microscopy, and metal ions detectable by their magnetic properties.

Some useful enzymatic labels include enzymes that cause a detectable change in a substrate. Some useful enzymes and their substrates include, for example, horseradish peroxidase (pyrogallol and o-phenylenediamine), beta-galactosidase (fluorescein beta-D-galactopyranoside), and alkaline phosphatase (5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium). The use of enzymatic labels have been described in U.K. 2,019,404, EP 63,879, and by Rotman, Proc. Natl. Acad. Sci., 47, 1981-1991 (1961).

Useful chromophores include, for example, fluorescent, chemiluminescent, and bioluminescent molecules, as well as dyes. Some specific chromophores useful in the present invention include, for example, fluorescein, rhodamine, Texas red, phycoerythrin, umbelliferone, luminol.

The labels may be conjugated to the antibody or nucleotide probe by methods that are well known in the art. The labels may be directly attached through a

functional gr up on the probe. The probe either contains or can be caused to contain such a functional group. Some examples of suitable functional groups include, for example, amino, carboxyl, sulfhydryl, maleimide, isocyanate, isothiocyanate.

The label may also be conjugated to the probe by means of a ligand attached to the probe by a method described above and a receptor for that ligand attached to the label. Any of the known ligand-receptor combinations is suitable. The biotin-avidin combination is preferred.

The polypeptide of the invention may be used to detect the presence of antibodies specific for N. meningitidis in a sample. The method comprises preparing a polypeptide containing a segment having an amino acid sequence that is substantially homologous to a member of the hemolysin family of toxins. The polypeptide may be prepared as described above. Preferably, the polypeptide comprises a segment having an amino acid sequence that is present in N. meningitidis.

The sample may, for example, be from a patient suspected of being infected with N. meningitidis. Suitable assays are known in the art, such as the standard ELISA protocol described by R.H. Kenneth, "Enzyme-Linked

Antibody Assay with Cells Attached to Polyvinyl Chloride Plates" in Kenneth et al, Monoclonal Antibodies, Plenum Press, N.Y., page 376 (1981).

Briefly, plates are coated with antigenic polypeptide at a concentration sufficient to bind detectable amounts of the antibody. After incubating the plates with the polypeptide, the plates are blocked with a suitable blocking agent, such as, for example, 10% normal goat serum. The sample, such as patient sera, is added and titered to determine the endpoint. Positive and negative controls are added simultaneously to quantitate the amount of relevant antibody present in the unknown samples. Following incubation, the samples are probed with goat anti-human Ig conjugated to a suitable enzyme. The presence of anti-polypeptide antibodies in the sample is indicated by the presence of the enzyme.

Antibodies raised against polypeptides of the present invention are capable of recognizing N. meningitidis and distinguishing meningococcal cells from gonococcal cells in a sample. The A4.85 monoclonal antibody, for example, recognizes a polypeptide expressed by iron-stressed meningococcal cells. A4.85 does not, however, recognize any proteins in iron-stressed N. gonorrhoeae.

The antibodies may be labelled by known methods as described above. Assays for distinguishing iron-stressed meningococcal cells from iron-stressed gonococcal cells follow known formats, such as standard blot and ELISA formats.

EXAMPLES

Example 1. Isolation of A4.85 MAb

Bacterial outer membranes are prepared from iron-stressed cultures of Neisseria meningitidis strain FAM20 as follows. FAM20 is inoculated into chelexed defined medium (CDM, West et al, J. Bacteriology 169, 3414 (1987)). This medium allows growth only until iron stores within the bacteria have been depleted. During this time, a set of proteins that are regulated by the availability of iron become expressed. Bacteria are harvested and outer membranes are prepared as described by Dyer et al in Infection and Immunity 56, 977 (1988).

Three to five BALB/c female mice are immunized with iron-stressed FAM20 outer membranes by either the intramuscular (im) or intraperitoneal (ip) routes. With the im route, 100 ug of antigen (Ag) is emulsified in complete Freund's adjuvant and injected on two different

sites on day zero, followed by booster doses two weeks apart with the Ag now emulsified in incomplete Freund's adjuvant. The ip route involves immunization with 100 ug of Ag on days zero, 7, 14 and 28. Serum antibody levels are checked by either ELISA or Western blotting three days following the final boost to determine serum antibody levels. Mice are given a final boost ip on each of three consecutive days before the fusion. On the day of the fusion, mice are sacrificed by cervical dislocation and the spleens are removed aseptically. Spleen cells are extracted by teasing the cells out of the sac using two bent 19 ga needles. Extracted cells are resuspended to give single cell suspensions.

Mouse myeloma cells, SP2.0-AG14 (ATCC CRL 1581), that have been grown in Dulbecco's Modified Eagle's Medium/Ham's F-12 (DMEM/F12) supplemented with 15% fetal calf serum (FCS), are used as the fusion partner. Cells are mixed in a 10:1 ratio of spleen:myeloma cells and pelleted together in a 50 ml centrifuge tube. The supernatant is aspirated off leaving a dry pellet to which 1 ml of 50% polyethylene glycol (PEG) (prewarmed to 37^{o}C) is added. The cells are gently resuspended and allowed to sit at room temperature for 2 minutes, after which 1 ml of DMEM/F12 without added sera is added and the cells gently resuspended. The cells are then further diluted and

resuspended with 2, 4, 8 and 16 ml of DMEM/F12 added at two-minute intervals. The cells are then pelleted and the supernatant aspirated. Two ml of DMEM/F12 supplemented with 15% FCS is carefully added to avoid resuspension of the pellet and then incubated for one hour at 37°C.

At the end of the 1 hour incubation, the suspension is diluted to a final concentration of $1x10^6$ cells/ml. This suspension is then poured into tissue culture flasks and incubated overnight. The next day an equal volume of culture media supplemented with 2X HAT (hypoxanthine, aminopterin, thymidine) components are plated out into 96-well plates with 200 ul/well with $1x10^5$ spleen cells/well. Plated cells are fed every 4-5 days after the fusion by aspiration of half the media from the wells and addition of fresh 1xHAT media. The wells are scored for growth after 10-14 days, and growing wells are tested for presence of secreted antibody by screening the culture supernatants by either an ELISA or Western blotting. Wells that prove positive on assay are expanded for growth into 24-well culture dishes in culture media with HT supplements (no aminopterin) and re-tested. Those proving positive on re-testing are expanded further into larger tissue culture vessels and then cloned twice by limiting dilution.

A cell line (A4.85) that arose from a single mouse spleen cell was isolated. A4.85 produces a monoclonal antibody (MAb) that reacts with several protein species (70 kilodaltons to several hundred kilodaltons in mass) on a Western blot of FAM20 outer membranes, each of whose synthesis is repressed by the presence of iron in the bacterial growth medium.

Example 2A. Isolation of Genomic Clones

A. Library construction

A library of Neisseria meningitidis strain FAM20 chromosomal DNA is constructed in the bacteriophage vector lambda-gt11 as follows. FAM20 chromosomal DNA is isolated by standard methods (Maniatis et al, 1982). The DNA is sheared by sonication to fragment sizes of approximately 300-1000 bp. Synthetic EcoRI linkers are ligated to the ends of these molecules, followed by cleavage with EcoRI restriction endonuclease to generate EcoRI restriction sites at the end of each molecule. The resulting fragments are ligated with EcoRI-cleaved lambda-gt11 DNA (Maniatis et al, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1982)). The ligated DNA is packaged into lambda phage heads using lambda packaging extracts (Promega

Corp., Madison, Wisconsin), according to manufacturer's instructions.

B. Library Screening and Isolation of DNA

The library created above is screened with the A4.85 MAb to detect clones that express the epitope recognized by A4.85. 500,000 recombinant plaques from the lambda-gt11 expression library are screened by the method of Maniatis et al (1982). A pure clone reacting with the A4.85 MAb is isolated by re-plating and screening the reactive plaque twice. The meningococcal insert DNA from the pure lambda clone (lambda 4.85) is amplified by the polymerase chain reaction (PCR) technique using a kit from Perkin-Elmer/Cetus. The PCR-amplified DNA is cloned into the sequencing vector M13mp19 (Maniatis et al, 1982) and the DNA sequence determined by the dideoxy chain termination method of Sanger et al (Proc. Natl. Acad. Sci. USA 74, 5463-5467 (19877)) using the Sequenase kit (Stratagene, La Jolla, CA).

The cloned meningococcal DNA is labelled with ^{32}Pby the random primed method with a kit from Boehringer-Mannheim (Indianapolis, IN) and is used in Southern hybridizations (Maniatis et al, 1982) to identify DNA restriction fragments in the FAM20 chromosome adjacent to

the DNA cloned in lambda 4.85. Chromosomal Sau3A I fragments of approximately 560 and 1600 bp hybridize to the cloned meningococcal DNA. FAM20 DNA is cleaved with Sau3A I and fractionated on a preparative agarose gel. Two size fractions are isolated, one of 400-700 bp and one of 1400-1800 bp.

The 560 bp Sau3A I fragment is cloned by ligating the 400-700 bp fraction of FAM20-Sau3A I fragments with BamHI-cleaved plasmid pBR322 (Maniatis et al, 1982). The desired clones of the 560 bp fragment are identified by hybridization of bacterial colonies containing recombinant plasmids with ^{32}P-labelledlambda 4.85 insert DNA (Maniatis et al, 1982). Plasmid DNA (pUNCH201) from a pure colony hybridizing with the DNA probe is prepared and its sequence determined using Sequenase as modified for use in double-strand sequencing (Kraft et al, BioTechniques 6, 544 (1988)). Southern hybridization is used to verify that the cloned fragment is representative of the fragment intact in the FAM20 genome.

To clone the 1600 bp fragment, the ends of the 1400-1800 bp fraction of FAM20-Sau3A I fragments are made blunt by reaction with Klenow enzyme and DNA nucleotides. Synthetic EcoRI linkers are added to these molecules, followed by ligation with EcoRI-cleaved, alkaline-

phosphatase-treated lambda ZAP DNA (Stratagene, LaJolla, CA) in accordance with technical information supplied with the lambda ZAP kit. Ligated DNA is packaged into lambda heads using Packagene lambda packaging extracts (Promega). The library of 1400-1800 bp FAM20-Sau3A I fragments is screened with a ^{32}P-labelledoligonucleotide (SAT1), which is synthesized to correspond to DNA sequences at one end of the lambda 4.85 insert (5′ GCCATTGCCACTGTAGATA 3′). A lambda ZAP plaque hybridizing with the SAT1 oligonucleotide is purified as above. The interior portion of this lambda ZAP clone (lambda ZAP202) is "excised" by the addition of helper bacteriophage. The excision results in a multicopy plasmid (pUNCH202) containing the cloned meningococcal insert. Southern hybridization is used to verify that the cloned fragment is representative of the fragment intact in the FAM20 genome. The sequence of the cloned DNA fragment is determined by double-strand sequencing as described above.

The adjoined sequence of pUNCH201, pUNCH202, and the lambda 4.85 insert reveals the presence of an open reading frame that contains the entirety of the cloned DNA. Neither the start or end of the gene is present within this cloned DNA. The DNA sequence is shown as Figure 1. The amino acid sequence predicted by the open reading

frame contains 835 amino acids (91kD). Th sequence is shown as Figure 2.

As determined by FASTA sequence comparison searches (see above), both the DNA and the deduced polypeptide sequence from this region have a high degree of similarity with a family of hemolysin bacterial toxins. For example, the DNA sequence shown in Figure I exhibits 54% identity with the cya gene (adenylate cyclase) from B. pertussis; 60% identity with the hlyA, hlyB, hlyC and hlyD gene from E. coli (hemolysin); 65% identity with hlyA, hlyB and hlyC gene (hemolysin) from E. coli; 56% identity with the leukotoxin gene from A. actinomycetemcomitans; 56% identity with the hemolysin gene from A. pleuropneumoniae; 60% identity with the leukotoxin gene from P. haemolytica; 62% identity with the A1 leukotoxin gene from P. haemolytica; and 57% identity with protease B gene of E. chrysanthemi.

The amino acid sequence predicted from the DNA sequence exhibited 25%-28% identity with leukotoxin, 22%-28% identity with hemolysin; and 30% identity with adenylate cyclase.

Meningococcal strain FAM20 contains at least two copies of DNA that encode the polypeptides of the

invention. This can be demonstrated by dig sting genomic DNA with the infrequent cutters BglII, SpeI, NheI, and combinations of NheI and SpeI. Southern blots of the digested DNA separated by pulse field gradient electrophoresis reveal two major bands that hybridize under stringent conditions to gene probes containing fragments of the sequence of the gene that encodes the polypeptide of the invention.

The remainder of the gene encoding the iron-regulated polypeptide of the invention is isolated in a manner similar to that described above for isolating pUNCH201 and pUNCH202. DNA restriction fragments either flanking the ends of the region already cloned or containing the entire region are identified by Southern hybridization using oligonucleotide probes derived from previously determined DNA sequence. These fragments are cloned into either plasmid or bacteriophage vectors as described above for pUNCH201 and pUNCH202. The DNA sequence of newly cloned fragments is determined as above, and reveals when either end of the gene is reached. If the gene is isolated on a single DNA fragment, it is expressed in an <u>in vitro</u> assay to verify that the protein that is encoded by this gene reacts with the A4.85 MAb. If the gene is not cloned intact on a single DNA fragment, it is reconstructed through standard molecular biology techniques to yield the

intact gene (Carbonetti, Proc. Natl. Acad. Sci. USA 84, 9084 (1987)).

For example, DNA fragments from one of the two copies of the structural genes coding for the polypeptide of the invention were purified from agarose gels, cloned and sequenced. Figure 3 shows the DNA sequence, which is complete in the 5' end of the gene. Underlined in Figure 3 are a typical promoter with a -35 and -10 region, a ribosome binding site, an ATG start site, and a consensus fur box, which is typically found in many gram negative iron-regulated promoters.

Example 2B. Western Blot and Molecular Weight

The full length polypeptide obtained from meningococcal strain FAM20 exhibits a molecular weight of 230-250 kD when subjected to Western blot analysis. Western blots may be carried out as follows:

Iron-starved whole cells of FAM20 are prepared in accordance with the method of West and Sparling, J. Bacteriol. 169, 3414-3421 (1987). The cells are washed in ice-cold Davis Minimal Medium A (Lederberg, Methods in Med. Res., 3:5 (1950)), immediately cooled on ice, and ruptured in a French pressure cell at 0°C and 20,000 psi.

The resulting mixture is centrifuged for 10 minutes at 20,000G, and the pellet solubilized in boiling SDS. Th solubilized membrane proteins are separated by standard 7.5% SDS-PAGE in Laemli buffer, which was described by Laemli in Nature 227, 680-685 (1970). The proteins are transferred (16 hours, 80 uA) onto Optibind nitrocellulose membranes (available from from Schleicher & Schuell). The membranes are blocked for 1 hour in 5% BSA in TBS (20mM Tris, 500 mM NaCl, pH 7.5); rinsed for 5 minutes in TBS; incubated for 1 hour with 1:2 dilution of monoclonal antibody A4.85 (see above) in 5% BSA; washed twice for 5 minutes in TBS and 0.05% Tween 20; incubated for 1 hour in a secondary antibody (goat anti-mouse lgG alkaline phosphatase conjugate) diluted in 5% BSA, available from BioRad (dilution = 1:3000) or Sigma (dilution = 1:1000); washed twice for 5 minutes in TBS/Tween; washed again for 5 minutes in TBS; and developed with an alkaline phosphatase substrate comprising 45ul Nitro Blue Tetrazolin, available from Sigma (75 mg/ml); 35 ul 5-bromo-4-chloro-3-indolylphosphate, p-tolnidine salt (50 mg/ml) in 10 ml of carbonate buffer, pH 9.8 (0.1 M $NaHCO_3$; 1mM $MgCl_2$).

Example 3. Assay for Antibody in Sample

A standard ELISA protocol is used to screen for the presence of antibodies against the polypeptide in proteins. Briefly, 96 well microtiter plates are coated with the antigen at concentrations varying from 50-1000ng per well in a high ph (9.6) carbonate buffer. The plates are incubated overnight at 9°C and blocked with 10% normal goat serum for one hour at 37°C. Patient sera is added and titered to determine the endpoint. Control positive and negative sera is added at the same time to quantitate the amount of relevant antibody present in the unknown samples. After a 2-3 hour incubation at 37°C, samples are probed with goat anti-human Ig conjugated to horseradish peroxidase. Positive samples are determined by using TMB.

The invention as claimed is enabled in accordance with the specification and readily available references and starting materials. Nevertheless, the following cell lines have been deposited in the American Type Culture Collection, Bethesda, Maryland on July 12, 1990 in order to facilitate the making and using of the invention:

Meningococcal cell line FAM18 (Accession Number 55071)

Meningococcal cell line FAM20 (Accession Number 55072)

Hybridoma cell line A4.85 (Accession Number HB 10504)

In addition, the following brochures containing useful protocols and information are available in the file history of this specification.

"Predigested Lambda Zap/Eco RI Cloning Kit Instruction Manual," Stratagene, La Jolla, California (November 20, 1987);

"Gigapack Plus" (for packaging recombinant lambda phage), Stratagene, La Jolla, California (April 25, 1988); and

"picoBlue Immunoscreening Kit" Instruction Manual," Stratagene, La Jolla, California (May 19, 1989).

CLAIMS

WHAT IS CLAIMED IS:

1. An isolated, antigenic polypeptide comprising a segment having at least fifty amino acid residues, wherein the amino acid sequence of the segment is present in N. meningitidis, and wherein the amino acid sequence is different from, but substantially homologous with, the amino acid sequence of a segment of a member of the hemolysin family of toxins, and antigenic fragments of such polypeptides.

2. The polypeptide of claim 1 wherein the segment has at least 100 amino acid residues.

3. The polypeptide of claim 1 wherein the segment has at least 200 amino acid residues.

4. The polypeptide of claim 1 in essentially pure form.

5. The polypeptide of claim 1 wherein the antigenic amino acid sequence is immunogenic.

6. The polypeptide of claim 1 wherein the amino acid sequence of the polypeptide comprises the sequence shown in Figure 2.

7. The polypeptide of claim 1 wherein antibodies against the polypeptide cross-react with at least one other member of the hemolysin family of toxins from other genera of bacteria.

8. The polypeptide of claim 1 wherein the other genera of bacteria include Escherichia, Serratia, Pasteurella, Proteus, Actinobacillus, and Bordetella.

9. The polypeptide of claim 7 wherein the other members of the hemolysin family of toxins comprise alpha-hemolysin from Escherichia coli; leukotoxin from Actinobacillus actinomycetemcomitans; leukotoxin from Pasteurella haemolytica; adenylate cyclase from Bordetella pertussis; and adenylate cyclase from Bacillus anthraces.

10. The polypeptide of claim 7 wherein the other member of the hemolysin family of toxins is alpha-hemolysin from E. coli.

11. A method of producing an antigen useful in protecting a mammal from infection by N. meningitidis comprising the steps of:

(a) preparing an isolated, antigenic polypeptide comprising a segment having at least fifty amino acid residues, wherein the amino acid sequence of the segment is different from, but substantially homologous with, the amino acid sequence of a segment of a member of the hemolysin family of toxins, or antigenic fragments of such polypeptides; and

(b) rendering the polypeptide or fragment non-toxic to mammals.

12. The method of claim 11 wherein the amino acid sequence is present in a polypeptide found in N. meningitidis.

13. The method of claim 11 wherein the mammal is a human.

14. A method of producing a vaccine composition useful in protecting a mammal from infection by N. meningitidis comprising the steps of:

(a) preparing an isolated, antigenic polypeptide comprising a segment having at least fifty amino acid residues, wherein the amino acid sequence of the segment is different from, but substantially homologous with, the amino acid sequence of a segment of a member of the hemolysin family of toxins, or antigenic fragments of such polypeptides; and

(b) rendering the polypeptide non-toxic to mammals; and

(c) combining the non-toxic polypeptide with a pharmaceutically acceptable carrier.

15. A method of claim 14 wherein the polypeptide is isolated from N. meningitidis.

16. The method of claim 14 wherein a the mammal is a human.

17. A vaccine composition capable of immunizing mammals against infections by N. meningitidis, the vaccine composition comprising:

(a) an immunogenic polypeptide that is non-toxic to mammals and comprises a segment having at least fifty amino acid residues, wherein the amino acid sequence is different from, but substantially homologous with, the amino acid sequence of a segment of a member of the hemolysin family of toxins, or antigenic fragments of such polypeptides; and

(b) a pharmaceutically acceptable carrier.

18. The vaccine composition of claim 17, wherein the polypeptide is present in N. meningitidis.

19. The vaccine composition of claim 17 wherein the polypeptide is present in outer membranes of N. meningitidis.

20. The method of claim 17 wherein the mammal is a human.

21. A method of immunizing mammals against infections by N. meningitidis comprising administering to mammals in need of such protection a vaccine composition that comprises an immunogenic polypeptide in a form that is non-toxic to mammals wherein the polypeptide comprises a segment having at least fifty amino acid residues, and wherein the amino acid sequence of the segment is substantially homologous with the amino acid sequence of a segment of a member of the hemolysin family of toxins, or antigenic fragments of such polypeptides.

22. The method of claim 21, wherein the amino acid sequence is present in N. meningitidis.

23. The method of claim 21 wherein the amino acid sequence is present in outer membranes of N. meningitidis.

24. The method of claim 21 wherein the mammal is a human.

25. Monoclonal antibodies raised against a polypeptide comprising a segment having at least fifty amino acid residues, wherein the amino acid sequence of the segment is present in N. meningitidis, and wherein the amino acid sequence of the segment is different from, but substantially homologous with, the amino acid sequence of a segment of a member of the hemolysin family of toxins, and antigenic fragments of such polypeptides.

26. An isolated nucleic acid molecule that encodes a polypeptide comprising a segment having at least fifty amino acid residues, wherein the amino acid sequence of the segment is present in N. meningitidis, and wherein the amino acid sequence is different from, but substantially homologous with, the amino acid sequence of a segment of a member of the hemolysin family of toxins, and antigenic fragments of such polypeptides.

27. A method of detecting the presence of antibodies specific for N. meningitidis in a sample comprising the steps of:

(a) preparing an isolated, antigenic polypeptide comprising a segment having at least fifty amino acid residues, wherein the amino acid sequence of the segment is substantially homologous with, the amino acid sequence

of a segment of a member of the hemolysin family of toxins, or antigenic fragments of such polypeptides; and

(b) determining whether the polypeptide recognizes an antibody in the sample.

28. The method of claim 27 wherein the polypeptide comprises an amino acid sequence present in N. meningitidis.

29. An isolated polypeptide comprising a segment having an amino acid sequence present in N. meningitidis wherein the amino acid sequence consists of at least three repeats of the nine amino acid hemolysin consensus sequence, the hemolysin consensus sequence consisting of at least four of:

L at position 1;

G at position 3;

G at position 4;

G at position 6;

N at position 7;

D at position 8; and

x at positions 2, 5 and 9;

wherein x represents any single amino acid residue, and antigenic fragments of such polypeptides.

30. The polypeptide of claim 29 wherein the segment has at least 100 amino acid residues.

31. The polypeptide of claim 29 wherein the segment has at least 200 amino acid residues.

32. The polypeptide of claim 29 in essentially pure form.

33. The polypeptide of claim 29 wherein the antigenic amino acid sequence is immunogenic.

34. The polypeptide of claim 29 wherein antibodies against the polypeptide cross-react with at least one other member of the hemolysin family of toxins from other genera of bacteria.

35. The polypeptide of claim 29 wherein the other genera of bacteria include Escherichia, Serratia, Pasteurella, Proteus, Actinobacillus, and Bordetella.

36. The polypeptide of claim 34 wherein the other members of the hemolysin family of toxins comprise alpha-hemolysin from Escherichia coli; leukotoxin from Actinobacillus actinomycetemcomitans; leukotoxin from Pasteurella haemolytica; adenylate cyclase from Bordetella pertussis; and adenylate cyclase from Bacillus anthracis.

37. The polypeptide of claim 34 wherein the other member of the hemolysin family of toxins is alpha-hemolysin from E. coli.

38. A method of producing an antigen useful in protecting a mammal from infection by N. meningitidis comprising the steps of:

(a) preparing an isolated polypeptide comprising a segment having an amino acid sequence that consists of at least three repeats of the nine amino acid hemolysin consensus sequence, the hemolysin consensus sequence consisting of at least four of:

L at position 1;

G at position 3;

G at position 4;

G at position 6;

N at position 7;

D at position 8; and

x at positions 2, 5 and 9;

wherein x represents any single amino acid residue; or antigenic fragments of such polypeptides.

(b) rendering the polypeptide or fragment non-toxic to mammals.

39. The method of claim 38 wherein the amino acid sequence is present in a polypeptide found in N. meningitidis.

40. A method of producing a vaccine composition useful in protecting a mammal from infection by N. meningitidis comprising the steps of:

(a) preparing an isolated, antigenic polypeptide comprising a segment having an amino acid sequence that consists of at least three repeats of the nine amino acid hemolysin consensus sequence, the hemolysin consensus sequence consisting of at least four of:

L at position 1;

G at position 3;

G at position 4;

G at position 6;

N at position 7;

D at position 8; and

x at positions 2, 5 and 9;

wherein x represents any single amino acid residue, or antigenic fragments of such polypeptides;

(b) rendering the polypeptide non-toxic to mammals; and

(c) combining the non-toxic polypeptide with a pharmaceutically acceptable carrier.

41. A method according to claim 40 wherein the polypeptide is isolated from N. meningitidis.

42. A vaccine composition capable of immunizing mammals against infections by N. meningitidis, the vaccine composition comprising:

(a) an isolated, immunogenic polypeptide comprising a segment having an amino acid sequence that consists of at least three repeats of the nine amino acid hemolysin consensus sequence, the hemolysin consensus sequence consisting of at least four of:

L at position 1;

G at position 3;

G at position 4;

G at position 6;

N at position 7;

D at position 8; and

x at positions 2, 5 and 9;

wherein x represents any single amino acid residue, or immunogenic fragments thereof; and

(b) a pharmaceutically acceptable carrier.

43. The vaccine composition of claim 42, wherein the polypeptide is present in N. meningitidis.

44. The vaccine composition of claim 43 wherein the polypeptide is present in outer membranes of N. meningitidis.

45. The method of claim 42 wherein the mammal is a human.

46. A method of immunizing mammals against infections by N. meningitidis comprising administering to mammals in need of such protection a vaccine composition that comprises an isolated, immunogenic polypeptide comprising a segment having an amino acid sequence that

consists of at least three repeats of the nine amino acid hemolysin consensus sequence, the hemolysin consensus sequence consisting of at least four of:

L at position 1;

G at position 3;

G at position 4;

G at position 6;

N at position 7;

D at position 8; and

x at positions 2, 5 and 9;

wherein x represents any single amino acid residue, or immunogenic fragments thereof.

47. The method of claim 46, wherein the amino acid sequence is present in N. meningitidis.

48. The method of claim 47 wherein the amino acid sequence is present in outer membranes of N. meningitidis.

49. The method of claim 46 wherein the mammal is a human.

50. Monoclonal antibodies raised against an isolated polypeptide comprising a segment having an amino acid sequence present in N. meningitidis wherein the amino acid

sequence consists of at least three repeats of the nine amino acid hemolysin consensus sequence, the hemolysin consensus sequence consisting of at least four of:

L at position 1;

G at position 3;

G at position 4;

G at position 6;

N at position 7;

D at position 8; and

x at positions 2, 5 and 9;

wherein x represents any single amino acid residue, or antigenic fragments of such polypeptides.

51. A method of treating mammals suffering from diseases caused by infection with N. meningitidis comprising the steps of:

(a) preparing antibodies specific to a polypeptide that is a member of the hemolysin family of toxins or an antigenic fragment thereof; and

(b) administering the antibodies to a mammal infected with N. meningitidis.

52. The meth d of claim 51 wherein the mammal is a human.

53. The method of claim 51 wherein the antibodies are raised against a polypeptide comprising a segment having an amino acid sequence present in N. 'meningitidis.

54. The method of claim 51 wherein the antibodies are monoclonal.

55. An isolated nucleic acid molecule that encodes a polypeptide comprising a segment having an amino acid sequence present in N. meningitidis wherein the amino acid sequence consists of at least three repeats of the nine amino acid hemolysin consensus sequence, the hemolysin consensus sequence consisting of at least four of:

L at position 1;

G at position 3;

G at position 4;

G at position 6;

N at position 7;

D at position 8; and

x at positions 2, 5 and 9;

wherein x represents any single amino acid residue, or antigenic fragments of such polypeptides.

56. A method of detecting the presence of N. meningitidis in a sample comprising the steps of:

(a) preparing a probe that recognizes a polypeptide that is a member of the hemolysin family of toxins or a fragment thereof, or a nucleic acid molecule encoding the polypeptide or fragment; and

(b) determining whether the probe recognizes N. meningitidis in the sample.

57. The method of claim 56 wherein the probe is an antibody.

58. The method of claim 57 wherein the antibody is monoclonal.

59. The method of claim 56 wherein the probe is a nucleic acid molecule.

60. The method of claim 56 wherein the polypeptide that is a member of the hemolysin family of toxins or nucleic acid molecule encoding the polypeptide is present in N. meningitidis.

61. A method of detecting the presence of antibodies specific for N. meningitidis in a sample comprising the steps of:

(a) preparing an isolated, antigenic polypeptide comprising a segment having an amino acid sequence that consists of at least three repeats of the nine amino acid hemolysin consensus sequence, the hemolysin consensus sequence consisting of at least four of:

L at position 1;

G at position 3;

G at position 4;

G at position 6;

N at position 7;

D at position 8; and

x at positions 2, 5 and 9;

wherein x represents any single amino acid residue, or antigenic fragments of such polypeptides; and

(b) determining whether the polypeptide recognizes an antibody in the sample.

62. The method of claim 61 wherein the polypeptide comprises an amino acid sequence present in N. meningitidis.

FIGURE 1: PARTIAL DNA SEQUENCE

```
   1  GAATTCCCTG AATGGGCAAG AGAGTGGTTG AAATTAGATC CCAAACGTTC
  51  AGGCAAATAT CATGTCTACG ACCCCCTCGC CCTAGATCTA GACGGCGACG
 101  GTATAGAAAC CGTTGCTGCC AAAGGCTTTG CAGGTGCATT GTTCGACCAC
 151  CGCAATCAAG GCATCCGCAC CGCCACCGGT TGGGTTTCTG CCGATGACGG
 201  TTTACTCGTC CGCGATTTGA ACGGCAACGG CATCATCGAC AACGGCGCGG
 251  AACTCTTCGG CGACAACACC AAACTGGCAG ACGGTTCTTT TGCCAAACAC
 301  GGCTATGCAG CTTTGGCCGA ATTGGATTCA AACGGCGACA ACATCATCAA
 351  CGCGGCAGAC GCCGCATTCC AAACCCTGCG TGTATGGCAG GATCTCAACC
 401  AGGACGGCAT TTCCCAAGCT AATGAATTGC GTACCCTTGA AGAATTGGGT
 451  ATCCAATCTT TGGATCTCGC CTATAAAGAT GTAAATAAAA ATCTCGGTAA
 501  CGGTAACACT TTGGCTCAGC AAGGCAGCTA TACCAAAACA GACGGTACAA
 551  CCGCAAAAAT GGGGGATTTA CTTTTAGCAG CCGACAATCT GCACAGCCGC
 601  TTCAAAGACA AAGTGGAACT CACTGCCGAA CAGGCAAAAG CCGCCAATCT
 651  TGCGGGCATC GGCCGTCTGC GCGATTTGCG CGAAGCTGCC GCATTGTCCG
 701  GCGATTTGGC CAATATGCTG AAAGCTTATT CTGCCGCCGA AACTAAAGAA
 751  GCACAGTTGG CATTGTTAGA TAATTTGATT CACAAATGGG CGGAAACCGA
 801  TTCGAACTGG GGCAAAAAAT CGCCAATGCG ACTTTCAACC GATTGGACGC
 851  AAACGGCTAA TGAAGGTATT GCACTGACAC CATCCCAAGT AGCACAACTA
 901  AAAAAGAACG CTTTAGTTTC CCTTTCTGAT AAAGCTAAAG CAGCTATTGA
 951  CGCCGCCCGC GACCGCATTG CCGTGCTTGA TGCCTACACG GGGCAGGATT
1001  CCAGCACACT CTATTACATG AGCGAAGAAG ACGCGCTTAA TATCGTCAAA
1051  GTAACCAACG ATACATACGA CCATCTCGCC AAAAACATCT ACCAAAACCT
1101  GTTGTTCCAA ACCCGTTTGC AGCCATATTT GAATCAAATC AGTTTCAAAA
1151  TGGAAAATGA TACGTTCACT TTGGATTTTA GTGGTCTTGT TCAAGCATTT
1201  AACCATGTCA AAGAAACTAA TCCGCAAAAA GCTTTTGTGG ATTTGGCCGA
1251  GATGCTTGCA TATGGCGAAC TTCGTTCTTG GTATGAAGGC CGAAGACTAA
1301  TGGCCGATTA TGTGGAGGAG GCAAAAAAAG CAGGTAAATT TGAAGATTAC
1351  CAGAAAGTGT TGGGTCAGGA GACCGTTGCA TTATTAGCTA AAACATCGGG
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FIGURE 1: CONTINUED

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1401  TACGCAAGCA GATGATATCC TGCAAAATGT AGGCTTTGGT CATAATAAAA
1451  ATGTTTCTTT ATATGGTAAT GACGGCAACG ACACTCTAAT CGGCGGTGCA
1501  GGCAATGATT ACTTGGAGGG CGGCAGCGGT TCGGATACTT ATGTCTTCGG
1551  CAAAGGCTTC GGTCAGGATA CGGTCTATAA TTACGACTAC GCTACCGGAC
1601  GCAAAGACAT CATCCGCTTT ACCGACGGTA TTACAGCCGA TATGCTGACT
1651  TTTACCCGAG AGGGCAACCA TCTTCTTATC AAGGCAAAAG ACGACAGTGG
1701  ACAAGTGACT GTTCAGTCCT ATTTCCAGAA CGATGGCTCA GGTGCTTACC
1751  GTATCGATGA GATTCATTTC GATAACGGCA AAGTACTGGA TGTTGCCACT
1801  GTCAAAGAAC TGGTACAGCA ATCCACCGAC GGTTCGGACA GATTGTATGC
1851  CTACCAATCC GGAAGTACCT TAAATGGCGG ATTGGGCGAT GACTATCTGT
1901  ACGGTGCCGA CGGGAATGAC CTGCTGAATG GTGATGCAGG CAACGACAGT
1951  ATCTACAGTG GCAATGGCAA TGATACGCTC GATGGAGGAG AAGGCAACGA
2001  CGCCCTGTAC GGCTATAATG GTAACGATGC ACTGAATGGT GGCGAAGGCA
2051  ATGATCATTT GAACGGCGAA GACGGTAACG ACACTCTGAT CGGCGGTGCC
2101  GGTAATGATT ACTTGGAGGG CGGCAGCGGT TCGGATACTT ATGTCTTCGG
2151  CAAAGGCTTC GGTCAGGATA CGGTCTATAA TTACGACTAC GCTACCGGAC
2201  GCAAAGACAT CATCCGCTTT ACCGACGGTA TTACAGCCGA TATGCTGACT
2251  TTTACCCGAG AGGGCAACCA TCTTCTTATC AAGGCAAAAG ACGGCAGTGG
2301  ACAAGTGACT GTTCAGTCCT ATTTCCAGAA CGATGGCTCA GGTGCTTACC
2351  GTATCGATGA GATTCATTTC GATAACGGCA AAGTACTGGA TGTTGCCACT
2401  GTCAAAAAAC TGGTACAGCA ATCCACCGAC GGTTCGGACA GATTGTATGC
2451  CTACCAATCC GGAAGTACCT TAAATGGCGG ATTGGGCGAT GACTATCTGT
2501  ACGGTGCCGA CGGGGATGAC CTGCTGAATG GTGATGCAGG CAACGACAGT
2551  ATCTACAGTG GCAATGGCAA TGATACGCTC AATGGAGGAG AAGGCAACGA
2601  CGCCCTGTAC GGCTATAATG GTAACGATGT ACTGAATGGT GCCGAAGGCA
2651  ATGATCATTT GAACGGCGAA GACGGTAACG ACACTCTAAT CGGCGGTGCA
2701  GGGCAATGAT TACTTGGAGG G
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FIGURE 2: PARTIAL AMINO ACID SEQUENCE

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  1  FPEWAREWLK LDPKRSGKYH VYDPLALDLD GDGIETVAAK GFAGALFDHR
 51  NQGIRTATGW VSADDGLLVR DLNGNGIIDN GAELFGDNTK LADGSFAKHG
101  YAALAELDSN GDNIINAADA AFQTLRVWQD LNQDGISQAN ELRTLEELGI
151  QSLDLAYKDV NKNLGNGNTL AQQGSYTKTD GTTAKHGDLL LAADNLHSRF
201  KDKVELTAEQ AYAANLAGIG RLRDLREAAA LSGDLANMLK AYSAAETKEA
251  QLALLDNLIK KWAETDSNKS KKSFMRLSTD WTQTANEGIA LTPSQVAQLK
301  KNALVSLSDK AYAAIDAARD RIAVLDAYTG QDSSTLYYMS EEDALNIVKV
351  TNDTYDHLAK NIYQNLLFCT RLQPYLNQIS FKMENDTFTL DFSGLVQAFN
401  HVKETNPQKA FVDLAEMLAY GELRSWYEGR RLMADYVEEA KKAGKFEDYQ
451  KVLGQETVAL LAKTSGTQAD DILQNVGFGH NKNVSLYGND GNDTLIGGAG
501  NDYLEGGSGS DTYVFGKGFG QDTVYNYDYA TGRKDIIRFT DGITADMLTF
551  TREGNHLLIK AKDDSGQVTV QSYFQNDGSG AYRIDEIHFD NGKVLDVATV
601  KELVQQSTDG SDRLYAYQSG STLNGGLGDD YLYGADGNDL LNGDAGNDSI
651  YSGNGNDTLD GGEGNDALYG YNGNDALNGG EGNDHLNGED GNDTLIGGAG
701  NDYLEGGSGS DTYVFGKGFG QDTVYNYDYA TGRKDIIRFT DGITADMLTF
751  TREGNHLLIK AKDGSGQVTV QSYFQNDGSG AYRIDEIHFD NGKVLDVATV
801  KKLVQQSTDG SDRLYAYQSG STLNGGLGDD YLYGADGDDL LNGDAGNDSI
851  YSGNGNDTLN GGEGNDALYG YNGNDVLNGA EGNDHLNGED GNDTLIGGAG
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FIGURE 3: PARTIAL DNA SEQUENCE INCLUDING NATURAL 5' END

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1451  TTATATGTCT TTATTTGAAT ATATCTTACG ATGGGGAAAT ATTTATATAT
1501  TTTATAATAA ATTTTACTCA TTTGCTAATA TGTCATGGAA TATTACTTGT
1551  ATTTTGTAGA ATTTTTCCAT ATGAAAATAT TCCATTTACT ATTTTTCTGA
1601  ACTTTATTAG TTTATTTTTA ATATTTTTAC CTCTTATATT TACCATAAGA
1651  GAGCTAATTG ATTCATATTA TATTGAGTCG ATAATTAATT TATTCTTAAT
1701  TTTAATTCCT CACGTTATTT TTTTAATTTA CTTGAAAGGA AAGCAGATAT
1751  GACATCTGCA AATTTTAATA TTAACGGTTT TGGAGATGTG AAATTAACAC
1801  CCTATTCACC ACTCTTGGGA TATAAAGCTT GGGATTCATT TATTGGTTCT
1851  ATTCAATCCT TATCTGATTT AATCTATAAT GTGGATAACA ATAGAAATAA
1901  AATGGAAATT ACTGTTAATA ATGCTATCCA AGCTGCAGAT AGCTTTTTAA
1951  GCAGTATTGG AAGAGATAAC AAAATAACAA ATACTGCTTC TTTACTTGCA
2001  TCCCTCGATA ACATTTTTTT AAATTTAAGA AATGTATCTC GAGATATACG
2051  AGAAACAGGA AAATTTAAAC CTAATGATAT TCAACAAGCA ATTGGTGATA
2101  TATTCATTGC TGCTGGTGAT GGATTACAAT ATATAAAACA ACAAACAGAG
2151  GCGATGGCTC AAAGCAAATT CTTACCAACT AAATTAAAAA CTGGTTTAAA
2201  TGATGTCCTT AATTCTAGAA TGCTAAAATC CTCTACTGTT TTACAGCATG
2251  AATTGAATTA TTTGGGATTT AAAATAAAGG ATTATGGAAA CGAGAGGCTT
2301  GGCGAATCTA TAATGAATAT AGATGATTTT ACACCAAGTA AGATAGCAAA
2351  CTTTTTTGCG GATCCTGATA CATACAGCAA TGTATTAGAA GAAGTATCTA
2401  GGTTTATATA TTCCTTAGTT CCTGATGATG CAAACCCTTG GAAAGGGGGC
2451  GAAGATTATA TTGGACGAGG GATAAGTGAA TGGGGAGAGT TACTGGAAAA
2501  ATGGTATAAA CAAGATTTTC TCCCTTATCT TGAAAAAGAA TGGGACCAAT
2551  TTCCGAAATT TGAAGATTGG CTGCCTGAAT TCCCTGAATG GGCAAGAGAG
2601  TGGTTGAAAT TAGATCCCAA ACGTTCAGGC AAATATCATG TCTACGACCC
2651  CCTCGCCCTA GATCTAGACG GCGACGGTAT AGAAACCGTT GCTGCCAAAG
2701  GCTTTGCAGG TGCATTGTTC GACCACCGCA ATCAAGGCAT CCGCACCGCC
2751  ACCGGTTGGG TTTCTGCCGA TGACGGTTTA CTCGTCCGCG ATTTGAACGG
2801  CAACGGCATC ATCGACAACG GCGCGGAACT CTTCGGCGAC AACACCAAAC
2851  TGGCAGACGG TTCTTTTGCC AAACACGGCT ATGCAGCTTT GGCCGAATTG
2901  GATTCAAACG GCGACAACAT CATCAACGCG GCAGACGCCG CATTCCAAAC
2951  CCTGCGTGTA TGGCAGGATC TCAACCAGGA CGGCATTTCC CAAGCTAATG
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FIGURE 3: CONTINUED

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3101  CAGCTATACC AAAACAGACG GTACAACCGC AAAAATGGGG GATTTACTTT
3151  TAGCAGCCGA CAATCTGCAC AGCCGCTTCA AAGACAAAGT GGAACTCACT
3201  GCCGAACAGG CAAAAGCCGC CAATCTTGCG GGCATCGGCC GTCTGCGCGA
3251  TTTGCGCGAA GCTGCCGCAT TGTCCGGCGA TTTGGCCAAT ATGCTGAAAG
3301  CTTATTCTGC CGCCGAAACT AAAGAAGCAC AGTTGGCATT GTTAGATAAT
3351  TTGATTCACA AATGGGCGGA AACCGATTCG AACTGGGGCA AAAAATCGCC
3401  AATGCGACTT TCAACCGATT GGACGCAAAC GGCTAATGAA GGTATTGCAC
3451  TGACACCATC CCAAGTAGCA CAACTAAAAA AGAACGCTTT AGTTTCCCTT
3501  TCTGATAAAG CTAAAGCAGC TATTGACGCC GCCCGCGACC GCATTGCCGT
3551  GCTTGATGCC TACACGGGGC AGGATTCCAG CACACTCTAT TACATGAGCG
3601  AAGAAGACGC GCTTAATATC GTCAAAGTAA CCAACGATAC ATACGACCAT
3651  CTCGCCAAAA ACATCTACCA AAACCTGTTG TTCCAAACCC GTTTGCAGCC
3701  ATATTTGAAT CAAATCAGTT TCAAAATGGA AAATGATACG TTCACTTTGG
3751  ATTTTAGTGG TCTTGTTCAA GCATTTAACC ATGTCAAAGA AACTAATCCG
3801  CAAAAAGCTT TTGTGGATTT GGCCGAGATG CTTGCATATG GCGAACTTCG
3851  TTCTTGGTAT GAAGGCCGAA GACTAATGGC CGATTATGTG GAGGAGGCAA
3901  AAAAAGCAGG TAAATTTGAA GATTACCAGA AAGTGTTGGG TCAGGAGACC
3951  GTTGCATTAT TAGCTAAAAC ATCGGGTACG CAAGCAGATG ATATCCTGCA
4001  AAATGTAGGC TTTGGTCATA ATAAAAATGT TTCTTTATAT GGTAATGACG
4051  GCAACGACAC TCTAATCGGC GGTGCAGGCA ATGATTACTT GGAGGGCGGC
4101  AGCGGTTCGG ATACTTATGT CTTCGGCAAA GGCTTCGGTC AGGATACGGT
4151  CTATAATTAC GACTACGCTA CCGGACGCAA AGACATCATC CGCTTTACCG
4201  ACGGTATTAC AGCCGATATG CTGACTTTTA CCCGAGAGGG CAACCATCTT
4251  CTTATCAAGG CAAAAGACGA CAGTGGACAA GTGACTGTTC AGTCCTATTT
4301  CCAGAACGAT GGCTCAGGTG CTTACCGTAT CGAT
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INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/05014

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): A61K 35/14; C07K 3/00; A61K 39/40; C12P 21/06, 21/04; C07H 15/12
U.S.Cl.: 530/387,350; 424/88; 435/69.1,71.1; 536/27

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.Cl.:	530/350; 424/88 , 71.1; 530/387; 536/27; 435/6, 7, 69.1

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

APS, Dialog files 155, 5, 50

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	Journal of Experimental Medicine, Volume 165, issued April 1987, Mietzner et al "Purification and Characterization of the Major iron-regulated Protein expressed by Pathogenic Neisseriae" pages 1041-1057, see entire article.	1-24, 29-49
Y	Infection and Immunity, Volume 51, No. 1, issued January 1986, Mietzner et al.," Distribution of an Antigenically Related Iron-regulated Protein among the Neisseria spp", pages 60-68, see entire article.	1-24, 29-49

* Special categories of cited documents: [10]
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
04 October 1991	14 NOV 1991
International Searching Authority	**Signature of Authorized Officer**
ISA/US	H. Sidberry

Form PCT/ISA/210 (second sheet) (Rev.11-87)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	Infection and Immunity, Volume 55, No. 12, issued December 1987, Strathdee et al.," Extensive Homology between the Leukotoxin of Pasteurella haemolytica A1 and the Alpha-Hemolysin of Escherichia coli," pages 3233-3236, see entire document.	1-24,
Y	Infection and Immunity, Volume 54, No. 3, issued December 1986, Black et al., "Human Immune Response to Iron-Repressible Outer Membrane Proteins of Neisseria Meningitidis," pages 710-713, see entire article.	5, 11-24, 33
Y	Infection and Immunity, Volume 58, No. 6, issued June 1990, Swihart et al., "The HpmA Hemolysin is more Common than HlyA among Proteus Isolates," pages 1853-1860, see pages 1853, Discussion page 1858.	29-39
Y	US,A, 4,681,761 (Mietzner et al) 21 July 1987, see entire document.	1-62
P,Y ✓	Infection and Immunity, Volume 58, No. 9, issued September 1990, Banerjee-Bhatnagar et al., "Expression of Neisseria Meningitidis Iron-regulated Outer Membrane Proteins, Including a 70-Kilodalton Transferrin Receptor, and their potential for Use as Vaccines," pages 2875-2881, see entire document.	1-62
P,Y ✓	Infection and Immunity, Volume 58, No. 9, issued September 1990, Pettersson et al., "Monoclonal antibodies against the 70-Kilodalton Iron-regulated Protein of Neisseria Meningitidis are Bactericidal and Strain specific", pages 3036-3041, see entire article.	25,50-54
Y	Infection and Immunity, Volume 57, No. 1, issued August 1989, Murakami et al., "Cloning and Characterization of the Structural Gene for the Class 2 protein of Neisseria meningitidis", pages 2318-2323, see entire article.	26,55